PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 25 February 2000 (25.02.00)	
International application No. PCT/GB99/01884	**Applicant's or agent's file reference** 38.46.68342/001
International filing date (day/month/year) 18 June 1999 (18.06.99)	**Priority date** (day/month/year) 18 June 1998 (18.06.98)
Applicant HAGERLID, Peter et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   18 January 2000 (18.01.00)

   [ ] in a notice effecting later election filed with the International Bureau on:

   _____

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Juan Cruz

Telephone No.: (41-22) 338.83.38

WO 99/66313
PCT/GB99/01884

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
PIÉSOLD, Alex, J.
Frank B. Dehn & Co.
179 Queen Victoria Street
London EC4V 4EL
ROYAUME-UNI

FILE 68342/001
30 DEC 1999
RECEIVED
ANSD
IMPORTANT NOTICE

Date of mailing (day/month/year)		
23 December 1999 (23.12.99)		
Applicant's or agent's file reference		
38.46.68342/001		
International application No.	**International filing date (day/month/year)**	**Priority date (day/month/year)**
PCT/GB99/01884	18 June 1999 (18.06.99)	18 June 1998 (18.06.98)
Applicant		
PYROSEQUENCING AB et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   EP,JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   None

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   23 December 1999 (23.12.99) under No. WO 99/66313

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/308 (July 1996)

3018162

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

Piesold, Alex J.
FRANK B. DEHN & CO.
179 Queen Victoria Street
London EC4V 4EL
GRANDE BRETAGNE

FILE 68342.001
18 SEP 2000
RECEIVED
ANSD

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	14.09.00
Applicant's or agent's file reference 38.46.68342/001	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/01884	18/06/1999	18/06/1998

Applicant
PYROSEQUENCING AB et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Conner, M Tel. +49 89 2399-2241

Form PCT/IPEA/416 (July 1992)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01884

I. **Basis of the report**

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-13 as originally filed

Claims, No.:

1-22 with telefax of 25/08/2000

Drawings, sheets:

1/3-3/3 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01884

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 5-9, 11-22
	No:	Claims 1-4, 10
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-22
Industrial applicability (IA)	Yes:	Claims 1-22
	No:	Claims

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB99/01884

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1.

Reference is made to the following documents :

D1: WO 95 22754 (VALTION TEKNILLINEN TUTKIMUSKESUS) 24 August 1995 (1995-08-24)

D2: EP-A-0 571 661 (PACKARD INSTRUMENT CO INC) 1 December 1993 (1993-12-01)

D3: WO 97 12678 A (CORNING COSTAR CORP) 10 April 1997 (1997-04-10)

D4: MARTIN C AND BRONSTEIN I: 'Imaging of Chemiluminescent Signals with Cooled CCD Camera Systems' JOURNAL OF BIOLUMINESCENCE AND CHEMILUMINESCENCE, vol. 9, 1994, pages 145-153, XP002116764

D5: "SEQ-light™ DNA sequencing system" (product information concerning apparatus used in D4); http://38.248.78.65/sqlkit.htm

D6: NICOLAS J: 'Applications of Low-Light Imaging to Life Sciences' JOURNAL OF BIOLUMINESCENCE AND CHEMILUMINESCENCE, vol. 9, 1994, pages 139-144, XP002116765

D7: WO 98 13523 A (DZIEGLEWSKA HANNA EVA ;PYROSEQUENCING AB (SE); UHLEN MATHIAS (SE);) 2 April 1998 (1998-04-02) cited in the application

D8 : EP-A-0 194 132 (DX CO LTD) 10 September 1986 (1986-09-10)

(Documents D1 and D5 were not cited in the International Search Report)

2.

The subject matter of claims 1 and 3 is not novel (Art. 33(2) PCT), for the following reasons :

2.1

Document D6 discloses (using terminology of claim 1) :

An apparatus (imaging luminometer) **for simultaneously monitoring an array of reaction sites** (see p. 139, col. 1, lines 12-15, D6) **for light indicating that a**

reaction is taking place at a particular site (see abstract, lines 5-8, D6), **comprising**:

- **means** (microtiter plate, see p. 139, col. 1, lines 4-7, D6) **for receiving a plurality of liquid samples at respective reaction sites** (wells of microtiter plate),
- **means for dispensing at least one reagent into said samples** (see p. 139, col. 1, lines 7-11, D6),
- **an optically sensitive device** (photon-counting camera, see p. 139, col. 1, lines 15-16 and col. 2, lines 1-8, D6) **arranged so that in use the light generated by the reaction of a particular liquid sample wil impinge upon a particular predetermined region of said optically sensitive device** (as is implicit for imaging systems, see col. 141, col. 2, 2nd paragraph, lines 12-15, D6),
- **means for determining the level of light implinging upon each of said predetermined regions** (see p. 139, col. 2, lines 7-11, D6);
- **means to record the variation of said light level with time for each of said liquid samples** (see p. 140, col. 1, '*Nucleic Acid Probes*', 2nd paragraph, lines 1-3, D6).

The subject matter of claim 1 is thus known from D6.

2.2

It can be added that the apparatus defined in claim 1 would not be considered inventive in view of D4 and D8, which both disclose an array of reaction sites (in particular a microtiter plate) monitored by a CCD camera (see the abstracts, D4, D8). All the features of claim 1 are thereby implied, except for reagent dispensing means. Such means are nevertheless well known in this art (see col. 1, first paragraph, D6) and a skilled person would consider it a normal option to incorporate them in a system according to D4 or D8, if not already present (see col. 2, second paragraph, D6).

2.3

Claim 3 defines an apparatus for identifying target bases in DNA sequences, presenting similar technical features as the apparatus defined in claim 1.

It is known from D7 to sequence DNA (see Abstract, last two lines, D7) in microtiter wells (see p. 8, last paragraph, D7) by measuring a light output according to claim 3 (see p. 2, second paragraph, D7) using a luminometer (see p. 4, fourth paragraph, and

p. 9, third paragraph, D7).

The imaging luminometer according to D6 has a sensitivity comparable to conventional luminometers (see Abstract, third paragraph, D6) and is therefore suitable for identifying target bases in DNA sequences following the method disclosed in D7.

The subject matter of claim 3 is therefore not novel for the same reasons as mentioned above in section 2.1.

3.

The subject matter of claim 18 is not inventive (Art. 33(3) PCT), for the following reasons :

3.1

Document D4 discloses (using terminology of claim 18) :

A method of identifying a target base in a DNA sequence (using the apparatus of Fig. 2, see also p. 149, last four lines of col. 1 and eleven first lines of col. 2; D4), **comprising :**

- detecting the light level emitted from a plurality of reaction sites (chemiluminescent blots) **on respective portions of an optically sensitive device** (CCD camera, see Fig. 4A and corresponding figure caption, D4),

- converting the light impinging upon each of said portions of said optically device into an electric signal which is distinguishable from the signals from all of said other regions (implicit when using a CCD camera),

- determining a light intensity for each of said discrete regions from the corresponding signal and recording said electric signals (see Fig. 4B and corresponding figure caption, D4).

3.2

The subject matter of claim 18 differs from D4 in that claim 18 defines "**recording the variations of said electric signals with time**", while D4 discloses intensity patterns recorded at a particular time (Fig. 7B, D4).

3.3
Recording said patterns with time is however an obvious step since more bases are added to the sequence information with time (see caption of Fig. 7B and also p. 152, lines 22-27, D4).

The subject matter of claim 18 is therefore not inventive in view of D4.

3.4
It can be added that the subject matter of claim 18 is also not inventive in view of a combination of D6 and D7. In effect, the method of sequencing DNA disclosed in D7 implies the steps defined in claim 18, except for the feature of detecting light levels from the reaction sites on **respective** portions of a light detector, which can be interpreted as the use of an imaging system, while D7 generally defines the use of a luminometer (see p. 9, third paragraph, D7).
A skilled person would find it obvious to use the imaging luminometer known from D6 to carry out the method known from D7, since said imaging luminometer is described in D6 as being of comparable sensitivity as conventional luminometers (see Abstract, D6) and further allows chemiluminescent data from microtitre wells to be acquired and processed much faster (see p. 139, col. 1, last two lines and col. 2, first three lines, D6). The skilled person would thereby arrive at the features of claim 18 without an inventive step being involved.

4.
Dependent claims 2, 4-17, 19-22 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty or inventive step, the reasons being as follows :

4.1
The subject matter of claims 2, 4 and 10 is not novel (Art. 33(2) PCT) :

- **claim 2** : see D6, p. 139, col. 1, second paragraph.
- **claim 4** : the imaging luminometer in D6 comprises a single photon-counting camera.
- **claim 10** : the photon-counting camera in D6 is suitable for recording the total light output from a given reaction of the microtitre plate, provided the optical arrangement

permits this.

4.2

The subject matter of claims 5-9, 11-17, 19-22 is not inventive (Art. 33(3) PCT) :

- **claim 5** : an arrangement to monitor the reaction sites from underneath in D6 would be obvious in order to avoid interference with the reagent dispersing means necessarily located above the reaction sites (see p. 139, col. 1, four last lines of paragraph 1, D6). An example of a microtiter plate monitored from below is also given in D1 (see Figs. 4a-b, D1).
- **claims 6, 8** : D6 mentions a CCD camera as possible imaging system (see p. 140, col. 2, last four lines of paragraph 2, D6; see also Fig. 2, D4) and D8 teaches the use of lensing systems in conjunction with CCD arrays (see p. 15, second paragraph, D8).
- **claim 7** : defines an a slight constructional change in a lensing system which comes within the scope of the customary practice followed by persons skilled in the art.
- **claim 9** : defines a known alternative CCD array.
- **claims 11-12** : define obvious means related to the use of a CCD array.
- **claims 13, 16** : it is known that temperature affects chemical reactions. Heat regulating means in the context of DNA sequencing are implicitely known from D5 (see p. 2, '*Advantages*', D5). Temperature-regulated microtitre plates are also obvious in this art.
- **claim 14** : see abstract and Fig. 2, D2 or abstract and Fig. 2, D3.
- **claim 15** : the upper plate (11, Fig. 1, D2) of microplate (10, Fig. 1, D2) is a single block provided with channels (open holes, see Fig. 5, D2) whose opaque walls (11, Fig. 5, D2) prevent adjacent light transmission.
- **claim 17** : defines a slight constructional change of the channels defined in claim 15 which would fall within the scope of customary practice followed by persons skilled in the art.
- **claim 20 (=19, see Re Item VIII)** : see Fig. 7, D4.
- **claim 20 (=21, see Re Item VIII)** : measuring the patterns of Fig. 7A, D4 with time (see above section 3.3) would obviously be done without adding reagents ("substrate", see legend of Fig. 7, D4) to the four consecutive initial blots defining the base lanes. The step defined in claim 20 (21) is also implicit in the method disclosed in D7 (see abstract, D7).
- **claim 22** : defines a method step implicitely known from D7 (see Figs. 1-8, D7).

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB99/01884

Re Item VII

Certain defects in the international application

The numbering of the claims is erroneous, starting from claim 20. In particular, claim 20 appears twice, with different subject matter. Moreover, the subject matter of second claim 20, resp. claim 21, is identical to that of claim 19, resp. first claim 20. It follows that :

- second claim 20 and claim 21 are not appropriately formulated as dependent claims,
- the dependency of claim 22 is erroneous.

Re Item VIII

Certain observations on the international application

With respect to Art. 6 PCT (clarity) : on top of p. 17 of the claim sheet, a portion of text corresponding to part of claim 18 appears, unrelated to the other claims and not understandable by itself.

PATENT COOPERATION TREATY

PCT

REC'D 18 SEP 2000
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
38.46.68342/001		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/01884	18/06/1999	18/06/1998
International Patent Classification (IPC) or national classification and IPC G01N21/76		
Applicant PYROSEQUENCING AB et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/01/2000	14.09.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bravin, M Telephone No. +49 89 2399 2417

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01884

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-13 as originally filed

Claims, No.:

1-22 with telefax of 25/08/2000

Drawings, sheets:

1/3-3/3 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 5-9, 11-22
	No:	Claims 1-4, 10
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-22
Industrial applicability (IA)	Yes:	Claims 1-22
	No:	Claims

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1.

Reference is made to the following documents :

D1: WO 95 22754 (VALTION TEKNILLINEN TUTKIMUSKESUS) 24 August 1995 (1995-08-24)

D2: EP-A-0 571 661 (PACKARD INSTRUMENT CO INC) 1 December 1993 (1993-12-01)

D3: WO 97 12678 A (CORNING COSTAR CORP) 10 April 1997 (1997-04-10)

D4: MARTIN C AND BRONSTEIN I: 'Imaging of Chemiluminescent Signals with Cooled CCD Camera Systems' JOURNAL OF BIOLUMINESCENCE AND CHEMILUMINESCENCE, vol. 9, 1994, pages 145-153, XP002116764

D5: "SEQ-light™ DNA sequencing system" (product information concerning apparatus used in D4); http://38.248.78.65/sqlkit.htm

D6: NICOLAS J: 'Applications of Low-Light Imaging to Life Sciences' JOURNAL OF BIOLUMINESCENCE AND CHEMILUMINESCENCE, vol. 9, 1994, pages 139-144, XP002116765

D7: WO 98 13523 A (DZIEGLEWSKA HANNA EVA ;PYROSEQUENCING AB (SE); UHLEN MATHIAS (SE);) 2 April 1998 (1998-04-02) cited in the application

D8 : EP-A-0 194 132 (DX CO LTD) 10 September 1986 (1986-09-10)

(Documents D1 and D5 were not cited in the International Search Report)

2.

The subject matter of claims 1 and 3 is not novel (Art. 33(2) PCT), for the following reasons :

2.1

Document D6 discloses (using terminology of claim 1) :

An apparatus (imaging luminometer) **for simultaneously monitoring an array of reaction sites** (see p. 139, col. 1, lines 12-15, D6) **for light indicating that a**

reaction is taking place at a particular site (see abstract, lines 5-8, D6), **comprising**:

- **means** (microtiter plate, see p. 139, col. 1, lines 4-7, D6) **for receiving a plurality of liquid samples at respective reaction sites** (wells of microtiter plate),
- **means for dispensing at least one reagent into said samples** (see p. 139, col. 1, lines 7-11, D6),
- **an optically sensitive device** (photon-counting camera, see p. 139, col. 1, lines 15-16 and col. 2, lines 1-8, D6) **arranged so that in use the light generated by the reaction of a particular liquid sample wil impinge upon a particular predetermined region of said optically sensitive device** (as is implicit for imaging systems, see col. 141, col. 2, 2nd paragraph, lines 12-15, D6),
- **means for determining the level of light implinging upon each of said predetermined regions** (see p. 139, col. 2, lines 7-11, D6);
- **means to record the variation of said light level with time for each of said liquid samples** (see p. 140, col. 1, '*Nucleic Acid Probes*', 2nd paragraph, lines 1-3. D6).

The subject matter of claim 1 is thus known from D6.

2.2

It can be added that the apparatus defined in claim 1 would not be considered inventive in view of D4 and D8, which both disclose an array of reaction sites (in particular a microtiter plate) monitored by a CCD camera (see the abstracts, D4, D8). All the features of claim 1 are thereby implied, except for reagent dispensing means. Such means are nevertheless well known in this art (see col. 1, first paragraph, D6) and a skilled person would consider it a normal option to incorporate them in a system according to D4 or D8, if not already present (see col. 2, second paragraph, D6).

2.3

Claim 3 defines an apparatus for identifying target bases in DNA sequences, presenting similar technical features as the apparatus defined in claim 1.

It is known from D7 to sequence DNA (see Abstract, last two lines, D7) in microtiter wells (see p. 8, last paragraph, D7) by measuring a light output according to claim 3 (see p. 2, second paragraph, D7) using a luminometer (see p. 4, fourth paragraph, and

p. 9, third paragraph, D7).

The imaging luminometer according to D6 has a sensitivity comparable to conventional luminometers (see Abstract, third paragraph, D6) and is therefore suitable for identifying target bases in DNA sequences following the method disclosed in D7.

The subject matter of claim 3 is therefore not novel for the same reasons as mentioned above in section 2.1.

3.
The subject matter of claim 18 is not inventive (Art. 33(3) PCT), for the following reasons :

3.1
Document D4 discloses (using terminology of claim 18) :

A method of identifying a target base in a DNA sequence (using the apparatus of Fig. 2, see also p. 149, last four lines of col. 1 and eleven first lines of col. 2; D4), **comprising** :

- **detecting the light level emitted from a plurality of reaction sites** (chemiluminescent blots) **on respective portions of an optically sensitive device** (CCD camera, see Fig. 4A and corresponding figure caption, D4),
- **converting the light impinging upon each of said portions of said optically device into an electric signal which is distinguishable from the signals from all of said other regions** (implicit when using a CCD camera),
- **determining a light intensity for each of said discrete regions from the corresponding signal and recording said electric signals** (see Fig. 4B and corresponding figure caption, D4).

3.2
The subject matter of claim 18 differs from D4 in that claim 18 defines "**recording the variations of said electric signals with time**", while D4 discloses intensity patterns recorded at a particular time (Fig. 7B, D4).

3.3
Recording said patterns with time is however an obvious step since more bases are added to the sequence information with time (see caption of Fig. 7B and also p. 152, lines 22-27, D4).

The subject matter of claim 18 is therefore not inventive in view of D4.

3.4
It can be added that the subject matter of claim 18 is also not inventive in view of a combination of D6 and D7. In effect, the method of sequencing DNA disclosed in D7 implies the steps defined in claim 18, except for the feature of detecting light levels from the reaction sites on **respective** portions of a light detector, which can be interpreted as the use of an imaging system, while D7 generally defines the use of a luminometer (see p. 9, third paragraph, D7).
A skilled person would find it obvious to use the imaging luminometer known from D6 to carry out the method known from D7, since said imaging luminometer is described in D6 as being of comparable sensitivity as conventional luminometers (see Abstract, D6) and further allows chemiluminescent data from microtitre wells to be acquired and processed much faster (see p. 139, col. 1, last two lines and col. 2, first three lines, D6). The skilled person would thereby arrive at the features of claim 18 without an inventive step being involved.

4.
Dependent claims 2, 4-17, 19-22 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty or inventive step, the reasons being as follows :

4.1
The subject matter of claims 2, 4 and 10 is not novel (Art. 33(2) PCT) :

- **claim 2** : see D6, p. 139, col. 1, second paragraph.
- **claim 4** : the imaging luminometer in D6 comprises a single photon-counting camera.
- **claim 10** : the photon-counting camera in D6 is suitable for recording the total light output from a given reaction of the microtitre plate, provided the optical arrangement

permits this.

4.2

The subject matter of claims 5-9, 11-17, 19-22 is not inventive (Art. 33(3) PCT) :

- **claim 5** : an arrangement to monitor the reaction sites from underneath in D6 would be obvious in order to avoid interference with the reagent dispersing means necessarily located above the reaction sites (see p. 139, col. 1, four last lines of paragraph 1, D6). An example of a microtiter plate monitored from below is also given in D1 (see Figs. 4a-b, D1).
- **claims 6, 8** : D6 mentions a CCD camera as possible imaging system (see p. 140, col. 2, last four lines of paragraph 2, D6; see also Fig. 2, D4) and D8 teaches the use of lensing systems in conjunction with CCD arrays (see p. 15, second paragraph, D8).
- **claim 7** : defines an a slight constructional change in a lensing system which comes within the scope of the customary practice followed by persons skilled in the art.
- **claim 9** : defines a known alternative CCD array.
- **claims 11-12** : define obvious means related to the use of a CCD array.
- **claims 13, 16** : it is known that temperature affects chemical reactions. Heat regulating means in the context of DNA sequencing are implicitely known from D5 (see p. 2, '*Advantages*', D5). Temperature-regulated microtitre plates are also obvious in this art.
- **claim 14** : see abstract and Fig. 2, D2 or abstract and Fig. 2, D3.
- **claim 15** : the upper plate (11, Fig. 1, D2) of microplate (10, Fig. 1, D2) is a single block provided with channels (open holes, see Fig. 5, D2) whose opaque walls (11, Fig. 5, D2) prevent adjacent light transmission.
- **claim 17** : defines a slight constructional change of the channels defined in claim 15 which would fall within the scope of customary practice followed by persons skilled in the art.
- **claim 20 (=19, see Re Item VIII)** : see Fig. 7, D4.
- **claim 20 (=21, see Re Item VIII) :** measuring the patterns of Fig. 7A, D4 with time (see above section 3.3) would obviously be done without adding reagents ("substrate", see legend of Fig. 7, D4) to the four consecutive initial blots defining the base lanes. The step defined in claim 20 (21) is also implicit in the method disclosed in D7 (see abstract, D7).
- **claim 22** : defines a method step implicitely known from D7 (see Figs. 1-8, D7).

Re Item VII

Certain defects in the international application

The numbering of the claims is erroneous, starting from claim 20. In particular, claim 20 appears twice, with different subject matter. Moreover, the subject matter of second claim 20, resp. claim 21, is identical to that of claim 19, resp. first claim 20. It follows that :

- second claim 20 and claim 21 are not appropriately formulated as dependent claims,
- the dependency of claim 22 is erroneous.

Re Item VIII

Certain observations on the international application

With respect to Art. 6 PCT (clarity) : on top of p. 17 of the claim sheet, a portion of text corresponding to part of claim 18 appears, unrelated to the other claims and not understandable by itself.

Claims

1. An apparatus for simultaneously monitoring an array of reaction sites for light indicating that a reaction is taking place at a particular site, comprising:

means for receiving a plurality of liquid samples at respective reation sites;

means for dispensing at least one reagent into said samples;

an optically sensitive device arranged so that in use the light generated by the reaction of a particular liquid sample will impinge upon a particular predetermined region of said optically sensitive device;

means for determining the level of light impinging upon each of said predetermined regions; and

means to record the variation of said light level with time for each of said liquid samples.

2. An apparatus as claimed in claim 1, wherein said means for receiving a plurality of liquid samples comprises a plate.

3. An apparatus for identifying target bases in DNA sequences comprising:

a plate for receiving a plurality of liquid samples at respective reaction sites;

means for dispensing at least one reagent into said samples;

an optically sensitive device arranged so that in use light generated by the reaction of a particular liquid sample signifying the incorporation of a nucleotide will impinge upon a particular region of said optically sensitive device;

means for determining the level of light impinging upon each of said said predetermined regions; and

means for recording the variation of said light level with time.

4. An apparatus as claimed in claim 1, 2 or 3, wherein the optically sensitive device comprises a single optical transducer.

5. An apparatus as claimed in claim 1, 2, 3 or 4, arranged to monitor the reaction sites from underneath.

6. An apparatus as claimed in any of claims 1 to 5, comprising an array of lenses between, or arranged in use between, said reaction sites and the optically sensitive device.

7. An apparatus as claimed in claim 6, wherein the lenses of said array are spaced by a smaller amount than the spacing of the corresponding reaction sites.

8. An apparatus as claimed in any preceding claim, wherein the optically sensitive device comprises a charge-coupled device.

9. An apparatus as claimed in claim 8, wherein the optically sensitive device comprises a frame transfer charge-coupled device.

10. An apparatus as claimed in any preceding claim, comprising means to record a measure of the total light output from a given reaction site.

11. An apparatus as claimed in any preceding claim, comprising means to convert the electrical output from said optically sensitive device into a digital signal.

12. An apparatus as claimed in claim 11, wherein said conversion means converts the signals from a plurality of neighbouring pixels in a single block.

13. An apparatus as claimed in any of claims 2 to 12,

wherein said plate is in contact with heat regulating means.

14. An apparatus as claimed in any of claims 2 to 13, wherein masking means are provided between reaction sites on the plate.

15. An apparatus as claimed in claim 14, wherein said masking means are provided by channels in a block.

16. An apparatus as claimed in claim 15, wherein said block comprises temperature regulating means.

17. An apparatus as claimed in claim 15 or 16, wherein said channels flare outwardly towards the lower part thereof.

18. A method of identifying a target base in a DNA sequence, comprising detecting the light level emitted from a plurality of reaction sites on respective portions of an optically sensitive device, converting the light impinging upon each of said portions of said optically sensitive device into an electrical signal which is distinguishable from the signals from all of said other regions, determining a light intensity for each of said discrete regions from the corresponding electrical signal, and recording the variations of said electrical signals with time.

19. A method as claimed in claim 18, comprising monitoring a plurality of reaction sites simultaneously.

20. A method as claimed in claim 18 or 19, wherein the interval between successive readings of the state of the optically sensitive device is less than or equal to the time between the addition of reagents to consecutive reaction sites.

which is distinguishable from the signals from all of said other regions, determining a light intensity for each of said discrete regions from the corresponding electrical signal, and recording the variations of said electrical signals with time.

21 ~~20~~. A method as claimed in claim 19, comprising monitoring a plurality of reaction sites simultaneously.

22 ~~21~~. A method as claimed in claim 19 or 20, wherein the interval between successive readings of the state of the optically sensitive device is less than or equal to the time between the addition of reagents to consecutive reaction sites.

23 ~~22~~. A method as claimed in any of claims 18 to 21 comprising recording the times at which a series of peaks in light output occur for each sample and, thereby enabling each peak to be associated with the addition of a particular reagent to the corresponding sample.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
38.46.68342/001		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/01884	18/06/1999	18/06/1998
Applicant		
PYROSEQUENCING AB et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 2

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/01884

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/76 C12Q1/68 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C12Q B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 948 975 A (ERWIN DAVID N ET AL) 14 August 1990 (1990-08-14) column 5, line 58 -column 7, line 15 column 10, line 25 - line 54; figures 8,10,11 ---	1
A	WO 98 13523 A (DZIEGLEWSKA HANNA EVA ;PYROSEQUENCING AB (SE); UHLEN MATHIAS (SE);) 2 April 1998 (1998-04-02) cited in the application abstract ---	2,18
A	EP 0 194 132 A (DX CO LTD) 10 September 1986 (1986-09-10) page 7, line 8 - line 31 page 9, line 27 -page 11, line 29 page 15, line 24 -page 16, line 26; figure 1 --- -/--	1,2,18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 September 1999	15/10/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Tabellion, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/01884

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	MARTIN C AND BRONSTEIN I: "Imaging of Chemiluminescent Signals with Cooled CCD Camera Systems" JOURNAL OF BIOLUMINESCENCE AND CHEMILUMINESCENCE, vol. 9, 1994, pages 145-153, XP002116764 abstract page 149, left-hand column; figure 6 ---	1,2,18
A	NICOLAS J: "Applications of Low-Light Imaging to Life Sciences" JOURNAL OF BIOLUMINESCENCE AND CHEMILUMINESCENCE, vol. 9, 1994, pages 139-144, XP002116765 page 139 -page 140 ---	1,2,18
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X	WO 97 12678 A (CORNING COSTAR CORP) 10 April 1997 (1997-04-10) abstract; figure 2 ---	14
X	EP 0 571 661 A (PACKARD INSTRUMENT CO INC) 1 December 1993 (1993-12-01) abstract; figures 1,5 -----	14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/01884

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